REVIEW ARTICLE

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Immunological Principles of Adverse Drug Reactions

The Initiation and Propagation of Immune Responses Elicited by Drug Treatment

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Abstract

Adverse drug reactions account for between 2 to 5% of all hospital admissions and can prevent the administration of an otherwise effective therapeutic agent. Hypersensitivity or immune-mediated reactions, although less common, tend to be proportionately more serious. There is convincing evidence to implicate the immune system in the pathogenesis of hypersensitivity reactions. Our understanding of the way in which the immune system recognises drugs is based on the hapten hypothesis; the onset of hypersensitivity involves drug bioactivation, covalent binding to proteins, followed by uptake, antigen processing and T cell proliferation. Central to this hypothesis is the critical role of drug metabolism, with the balance between metabolic bioactivation and detoxification being one important component of individual susceptibility.

The purpose of this review is to classify drug hypersensitivity reactions in terms of their clinical presentation, and also to consider recent advances in our understanding of the chemical, biochemical and, in particular, cellular immunological mechanisms of hypersensitivity. The following topics are reviewed: (i) drug dis-

position and cellular metabolism; (ii) mechanisms of antigen processing and presentation; (iii) the role of cytokines and co-stimulatory molecules in the induction and maintenance of a polarised immune response; and (iv) the application of the hapten hypothesis, danger hypothesis and serial triggering model to drug hypersensitivity. A greater understanding of the mechanism(s) of hypersensitivity may identify novel therapeutic strategies and help to combat one of the more severe forms of adverse reactions to drugs.

Despite vigorous preclinical and clinical trials and mechanistic advances in our understanding of chemical toxicology and molecular biology, adverse drug reactions remain a major clinical problem. They account for between 2 to 5% of hospital admissions and can prevent the administration of an otherwise effective therapeutic agent.[1-3] It has been estimated that adverse drug reactions account for approximately 100 000 deaths per annum in the US[3] and increase the length of hospital stay and costs.[4]

Adverse drug reactions can be classified in terms of their clinical, pharmacological and chemical characteristics. Table I presents one such classification, with the characteristics of the different types of reactions.[5] Many but not all type B reactions have an immune pathogenesis, and are the focus of this review. Adverse reactions which fall into the types A, C, D and E categories, although important in terms of their frequency, and ability to cause morbidity and mortality, will not be considered any further because they are not immune-mediated.

Type B adverse reactions cannot be predicted from the known pharmacology of the drug, and do not show a simple dose-response relationship. Type A adverse reactions are quantitatively the most important type of adverse drug reaction. However, the importance of type B reactions lies in the fact that they are proportionately more serious than type A reactions, and account for many deaths. They are also termed idiosyncratic reactions: this is a functional term, which does not imply any specific aetiology, but signifies that the mechanisms are not well understood.[6,7] However, there is increasing evidence to implicate drug metabolism and the immune system in the pathogenesis of many of these reactions (fig. 1).[8-10] Nevertheless, many questions remain unanswered or only partly answered, such as why the reactions only occur in a minority of indi-

Table I. Classification of adverse drug reactions (reproduced from Park et al.,[5] with permission)

Type A (augmented) reactions
Reactions which can be predicted from the known pharmacology of the drug. These reactions are dose-dependent and can be alleviated by a dose reduction. Examples include bleeding with anticoagulants and bradycardia and heart block with β-blockers
Type B (bizarre) reactions
Reactions which cannot be predicted from the known pharmacology of the drug. These reactions do not show any dose-response relationship and host-dependent factors seem to be important in predisposition, although for the most part these have not been elucidated. Such reactions have a metabolic and/or immunological component which may determine individual susceptibility. Examples include halothane hepatitis and anticonvulsant hypersensitivity
Type C (chemical) reactions
Reactions whose biological characteristics can be either predicted, or clearly rationalised, in terms of the chemical structure of the drug (metabolite). Examples include paracetamol (acetaminophen) hepatotoxicity and ipomeanol pulmonary toxicity
Type D (delayed) reactions
Adverse reactions that occur after many years of treatment. Examples include secondary tumours many years after treatment with chemotherapeutic agents and teratogenic effects seen in children after drug intake by the mother during pregnancy (e.g. fetal hydantoin syndrome with phenytoin)
Type E (end-of-treatment) reactions
Adverse reactions that occur on drug withdrawal, especially when the drug is stopped suddenly. Examples include withdrawal seizures on stopping phenytoin and a withdrawal syndrome on stopping paroxetine

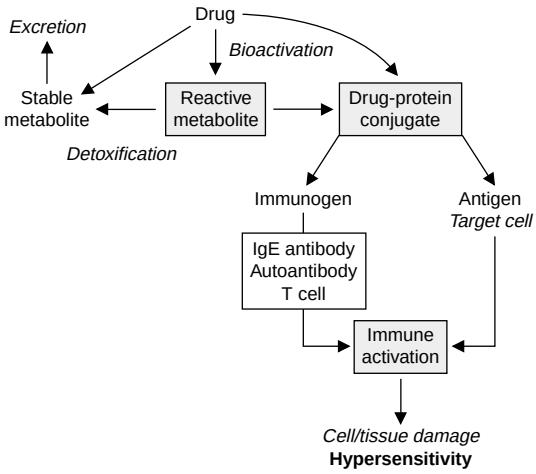

Fig. 1. Postulated mechanism for drug hypersensitivity (type B) reactions. **IgE** = immunoglobulin E.

viduals, what the individual predisposing factors are, as well as the mechanism by which a small molecule can lead to a systemic, and potentially fatal immune reaction. Hence, the purpose of this review is to provide a state-of-the-art appraisal of our current knowledge of how drugs can induce immune-mediated reactions. Such reactions are also called hypersensitivity reactions.[11,12]

In this review, we reserve the term hypersensitivity for adverse reactions that are characterised by a specific unwanted immune-mediated allergic reaction, which results in tissue damage. Other authors have used the term hypersensitivity to imply the occurrence of any adverse reaction, irrespective of whether it has an immune aetiology or not.[13] We feel that this is not a correct use of the term, since it is likely to cause confusion, given that there is a long-standing classification of hypersensitivity reactions of immune aetiology devised by Gell and Coombs that is widely accepted and used.[14]

1. Clinical Classification of Drug Hypersensitivity

The primary function of the immune system is to recognise and initiate rapid and specific responses against nonself antigens. Hypersensitivity reactions result from the immune system responding in an appropriate manner to an inappropriate stimulus. Based on clinical manifestations of tissue damage following the administration of a toxic chemical, Coombs and Gell[14] classified hypersensitivity reactions in 4 categories (fig. 2). Although this classification has been overtaken by recent advances in our understanding of cellular/molecular biology and in particular cellular immunology, the classification does begin to provide a framework to investigate the mechanisms of such reactions.

The classes of hypersensitivity are not mutually exclusive. For example, clinical characteristics and laboratory investigations place penicillin in all 4 categories of the Coombs and Gell classification. The β-lactam ring which is the common structural moiety of all penicillins is chemically reactive per se and can be a target for nucleophilic attack by free lysine groups of proteins.[15] Penicillin can also rearrange to penicillenic acid, which is more reactive and more immunogenic than penicillin itself (fig. 3).[16] Although the degree of covalent binding is not a determinant of the type of immune response,[17] the structure and function of the target protein may be important.[18] Covalently-linked penicilloyl-protein conjugates play a role in over 75% of immunoglobulin (Ig) E-mediated (type I) hypersensitivity reactions and have been implicated in type II to IV reactions, leading to blood dyscrasias, serum sickness–like symptoms and skin rashes, respectively.[19,20] For this reason, it is better to classify hypersensitivity reactions in terms of their clinical presentation (i.e., anaphylaxis, blood dyscrasias, vasculitis, hepatic, renal and pulmonary reactions, skin eruptions, immune-complex disease, autoimmunity) and then consider the role of either specific antibodies (IgE, IgG or IgM) or cytotoxic T cells and/or inflammatory cells in the pathogenesis of the adverse reaction. Drugs commonly associated with hypersensitivity are shown in table II.[21-25]

1.1 Anaphylaxis

Anaphylaxis is an immediate reaction following secondary exposure to an antigen. Anaphylaxis is relatively rare, the onset of symptoms is rapid and po-

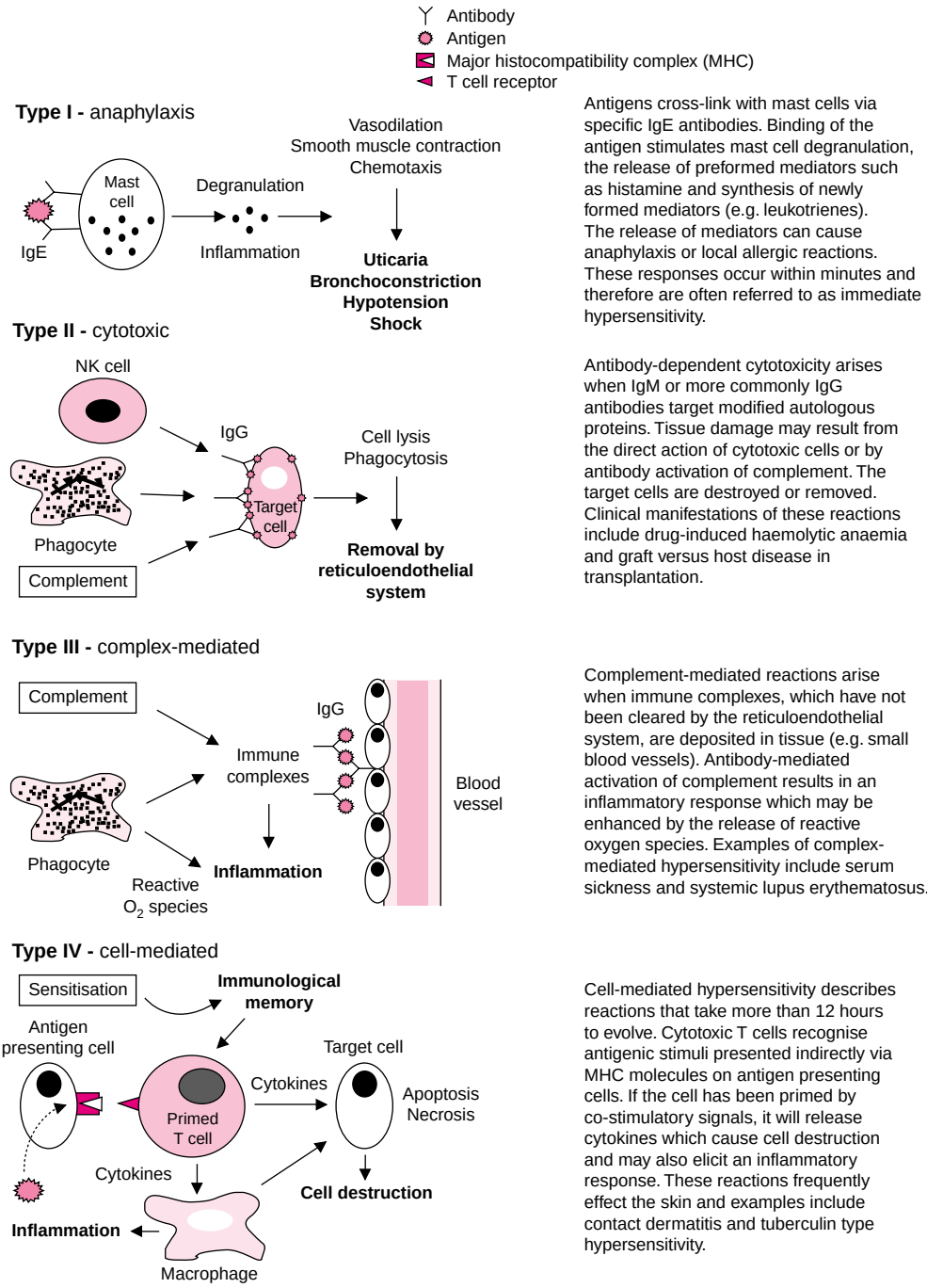

Fig. 2. Classification of hypersensitivity reactions according to Coombs and Gell (reproduced from Park et al.,[5] with permission). **Ig** = immunoglobulin; **NK** = natural killer.

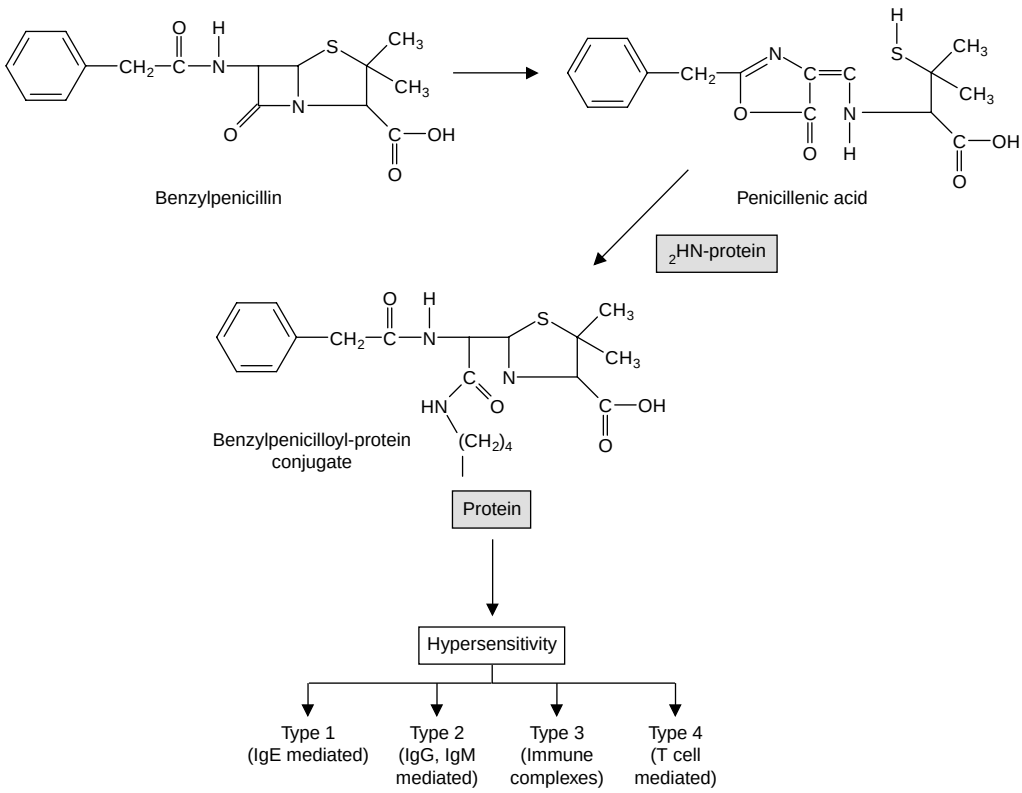

Fig. 3. Structure of benzylpenicillin and postulated mechanism of protein conjugation of the antigenic determinant. **Ig** = immunoglobulin.

tentially fatal.[26] Fatalities associated with penicillin-induced anaphylaxis have an incidence of 0.0015 to 0.002%.[27] Sensitised individuals produce IgE antibodies which bind to high-affinity receptors on mast cells and basophils. Receptor binding stimulates degranulation, release of mediators such as histamine and the activation of cytokine gene transcription.[28] Histamine release causes vasodilatation, blood pressure drops resulting ultimately in the rapid onset of anaphylaxis. A high incidence of anaphylaxis has resulted in the withdrawal of at least 4 drugs, the most recent being temafloxacin in 1992.[19] Temafloxacin was withdrawn because of potentially fatal anaphylaxis, haemolytic anaemia, hypoglycaemia, hepatic dysfunction and renal failure.

1.2 Blood Dyscrasias

Although uncommon, blood dyscrasias are often serious adverse reactions. Drugs may affect either pluripotential stem cells resulting in aplastic anaemia,[29] neutrophil precursors/neutrophils resulting in agranulocytosis[30] or erythrocytes resulting in haemolytic anaemia.[31] Haemolytic anaemia following high doses of penicillin to patients and experimental animals is associated with the formation of drug-specific IgG and IgM antibodies and subsequent complement activation.[32,33] More recently, diclofenac has been shown to cause haemolytic anaemia by formation of an intermediary glucuronide metabolite.[34] Nomifensine is an example of a drug that was withdrawn because of immune-mediated haemolytic anaemia.

Table II. Drugs which cause hypersensitivity reactions in humans[5]

Anaphylaxis	Blood dyscrasias	Hepatotoxicity	Cutaneous toxicity
Aspirin (acetylsalicylic acid)	Aminophenazone	Alcohol (ethanol)	Carbamazepine
Cefalosporins	Amodiaquine	Amineptine	Chlormezanone
Penicillins	Captopril	Amodiaquine	Dapsone
Streptokinase	Chlorpromazine	Carbamazepine	Lamotrigine
Sulfamethoxazole	Mianserin	Diclofenac	Lidocaine (lignocaine)
Suxamethonium	Penicillins	Dihydralazine	Penicillins
Thiopental	Sulfamethoxazole	Halothane	Phenobarbital (phenobarbitone)
Trimethoprim	Sulfasalazine	Phenytoin	Phenytoin
Tubocurarine	Valproic acid (sodium valproate)	Tienilic acid	Sulfamethoxazole

In general, drugs that cause agranulocytosis are also associated with aplastic anaemia, although agranulocytosis is more common.[35] Agranulocytosis is characterised by a depletion of neutrophils to below 0.5 x 10^9 cells/L. Aminophenazone (aminopyrine), amodiaquine and mianserin are examples of drugs that are associated with a relatively high incidence of agranulocytosis.[36-38] The clinical picture is an acute onset of fever caused by massive destruction of neutrophils and release of pyrogens, sore throat, and a variety of infections. The time-course of symptoms, especially on secondary exposure to the drug, are strongly suggestive of an immune pathogenesis. There is also laboratory evidence that the reactions are immune-mediated:

- Serum from an aminophenazone-sensitive patient has been shown to inhibit colonies cultured from mononuclear cells in the presence of the drug, while serum from controls did not.[39]
- IgG antibodies have been detected in patients administered amodiaquine.[40]
- Serum from mianserin-sensitive patients has been shown to produce complement and drug-dependent lysis of granulocytes.[41]

The use of clozapine, an atypical antipsychotic, has also been restricted because of a relatively high incidence (0.8%) of agranulocytosis.[42] Administration of clozapine is contingent upon a patient monitoring scheme in which the neutrophil count has to be monitored weekly, twice weekly or monthly, depending on the time of treatment and the stability of the haematological profile. The pathogenesis of clozapine-induced agranulocytosis is thought to be caused by direct effects on cell function (i.e. apoptosis or necrosis)[43,44] and the involvement of the immune system has been largely excluded.[45,46]

1.3 Hepatic Reactions

Many drugs are known to cause liver damage that varies in severity from mild increases in serum transaminase levels to fulminant hepatic failure. There are several examples of severe 'immunoallergic hepatitis' (table II).[6] Halothane, which can be considered a model for immune hepatitis, shows 2 forms of liver damage. First, a mild increase in serum transaminases which is thought to have a direct aetiology (i.e. binding of the metabolite to protein affects its function); and secondly, halothane hepatitis which is characterised by severe liver cell necrosis.[47] Halothane hepatitis occurs in only 1 in 35 000 patients on primary exposure, and 1 in 3700 on secondary exposure.[48] Drug-related antibodies, auto-antibodies and mononuclear cell infiltrates have been identified in sensitised patients,[49-51] whereas no antibodies or immune cells were found in patients who did not experience liver damage.[49,51] An animal model investigating the immune mechanisms of halothane hepatitis provides strong evidence that a cellular response is involved. Kupffer cells from halothane-exposed guinea pigs take up and present trifluoroacetylated protein adducts to lymphocytes in the liver resulting in T cell proliferation.[52,53] Tienilic acid and diclofenac are also associated, in rare cases, with chronic hepatitis.[54,55] Liver damage is accompanied by the presence of symptoms such as fever, eosinophilia and sore throat, suggesting an immunological mechanism.[54,56] In addition, animal models of diclofenac hepatitis have

implicated both humoral and cellular immunity in the pathogenesis of hypersensitivity.[57,58]

1.4 Cutaneous Eruptions

A recent study analysing adverse drug reactions reported to the spontaneous surveillance systems in 4 Italian regions revealed that the skin was the most frequently affected organ.[59] Drug-induced skin reactions affect between 2 to 3% of all hospitalised patients.[60] Fortunately, severe skin reactions (Stevens-Johnson syndrome and toxic epidermal necrolysis) represent only 1 in 1000 cases of all cutaneous forms of hypersensitivity. Sulfonamides, penicillins, anticonvulsants and nonsteroidal anti-inflammatory drugs are commonly associated with skin eruptions.[19,61] Severe skin rashes tend to occur during the first 4 weeks of treatment and the majority of cases are accompanied by fever, lymphadenopathy and eosinophilia.[10,62,63] Immunohistological signs include a dermal and epidermal infiltrate of helper CD4+ and cytotoxic CD8+ T lymphocytes, respectively.[64,65] These findings and the detection of drug-specific T cells from hypersensitive individuals that lyse autologous target cells by the perforin pathway provide direct evidence that T cells are involved in severe cutaneous eruptions.[66,67]

2. The Relationship Between Drug Disposition and Hypersensitivity

In nature, electrophilic 'chemically reactive' agents tend to be toxic.[68] Certain drugs (e.g. penicillins, cefalosporins and captopril) react directly with cellular or serum proteins.[24,25] Most drugs however, are not directly chemically reactive but through the normal process of drug metabolism, chemically reactive and potentially toxic intermediates may be generated. Drug metabolism is classified into phase I and phase II reactions. Phase I are oxidation and reduction reactions that usually form water soluble products. Phase II are conjugation reactions that involve the coupling of a drug (or phase I metabolite) with a polar group (e.g. glutathione, glucuronic acid). Formation of chemically reactive metabolites, or drug bioactivation, is usually catalysed by cytochrome P450 enzymes, which are quantitatively the most important group of enzymes involved in this process. Cytochrome P450 enzymes are present in many organs in the body, including the liver[69] and thus can bioactivate drugs to cause organ-specific hypersensitivity.[35,70-73]

Metabolism of drugs by phagocytes (monocytes, macrophages, neutrophils and Langerhans cells) is also of clinical importance. Phagocytes metabolise structurally unrelated compounds by the actions of the myeloperoxidase/ nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system and to a lesser extent cytochrome P450 enzymes.[35,74,75] Thus, phagocytes may provide a link between the formation of a chemically reactive metabolite and stimulation of a cellular immune response. Table III lists several drugs that have been shown to cause hypersensitivity through the formation of reactive metabolite(s).[76-86] The list, which is by no means exhaustive, should be regarded with some caution as much of the evidence supporting the role of drug bioactivation in the pathogenesis of hypersensitivity is indirect, probably due to the instability of reactive intermediates and/or technical difficulties in the characterisation of cellular responses *in vitro*.

The balance between drug bioactivation and detoxification may be one important determinant of drug hypersensitivity (fig. 1). In the majority of individuals, formation of a reactive metabolite is counterbalanced by the actions of glutathione (the most abundant intracellular antioxidant)[87] and detoxification enzymes.[88-90] However, infection, concomitant drug therapy and immune dysregulation have all been postulated to increase the production and/or decrease the elimination of chemically reactive metabolites.[9,71,91-95] Thus, certain individuals have the potential to generate large quantities of reactive metabolites, even at low dosage regimens.

Reactive metabolites haptenate biological macromolecules, usually serum or cellular proteins. Drug-protein conjugates are recognised by the immune system, processed and presented to the effector arm of the immune response. These processes

Table III. Examples of drugs that undergo metabolic activation and cause hypersensitivity in humans

Reaction	Drug	Reactive metabolite	References
Blood Dyscrasias	Aminophenazone (aminopyrine)	Dication	76
	Amodiaquine	Quinoneimine	77
	Mianserin	Iminium ion	78
Hepatotoxicity	Halothane	Acyl halide	79
	Diclofenac	Acyl glucuronide	57
	Tienilic acid	Sulfoxide	80
Cutaneous Reactions	Carbamazepine	Epoxide/quinone	81,82
	Phenytoin	Epoxide/quinone	83,84
	Sulfamethoxazole	Hydroxylamine/nitroso	85,86

may lead to either specific immunity to the modified protein and/or breakdown of tolerance and an altered response to 'self' proteins. The presently accepted mechanism by which drugs initiate hypersensitivity is based on the hapten hypothesis of immune recognition, initially described by Landsteiner and Jacobs.[96] The remainder of this review will detail our current understanding of the chemical, biochemical and cellular immunological mechanisms of drug hypersensitivity in humans.

3. The Hapten Hypothesis of Immune Recognition: The Role of Drug-Protein Conjugation

The acquired immune system is governed by an interaction between a T cell and a drug-conjugated peptide in the groove of a major histocompatibility complex [MHC; human leucocyte antigen(HLA)] molecule on an antigen presenting cell. In the presence of the appropriate co-stimulatory signals, immune recognition and stimulation of an immune response can result in hypersensitivity.[97-99] Generation of immune effectors depends on the route of administration, dose and physico-chemical properties of the drug, the degree and site of drug metabolism, and pathway of immunological processing once the drug-protein conjugate is recognised by the immune system.[5,17,100-102] Such reactions are usually divided into 3 stages: chemical activation, immune recognition and elicitation of an immune response.

In general, the immune system recognises molecules with a molecular weight above 1000D.[103] Therefore, the majority of drugs, with a molecular weight below 1000D are not immunogenic *per se*. For a drug to generate a full antigen and stimulate an immune response, it must conjugate a large molecular weight carrier molecule. This hypothesis termed 'the hapten theory of immune recognition of drugs by T cells' is based on classical experiments with model haptens.[5,10,13,96,104-106] Central to this hypothesis is the critical role of drug metabolism.[8,107-109] Although the normal process of drug metabolism results in detoxification, oxidation of certain drugs can lead to the generation of chemically reactive metabolites and protein haptenation. The immune system recognises 2 types of chemical antigen: one where the hapten (commonly a chemically reactive metabolite) is recognised by the antigen presenting cell and the other where the drug-modified peptide is antigenic.

Immune recognition occurs when antigen presenting cells uptake and process antigenic moieties, migrate to regional lymph nodes and trigger naive T cells expressing $\alpha\beta$-antigen receptors. Clonal expansion leads to the production of long-lived antigen-specific memory T cells. On subsequent exposure, antigen presenting cells recognise, process and present the antigen to memory T cells. Stimulated T cells proliferate and secrete cytokines that enhance and sustain the immune response (fig. 4).[110-112] Cytokines are soluble proteins that act as chemical communicators between cells. Binding of cytokines to specific receptors on the surface of target cells stimulates signal transduction and second messenger pathways.

Antigen presenting cells take up and process indiscriminately and do not recognise specific drug-

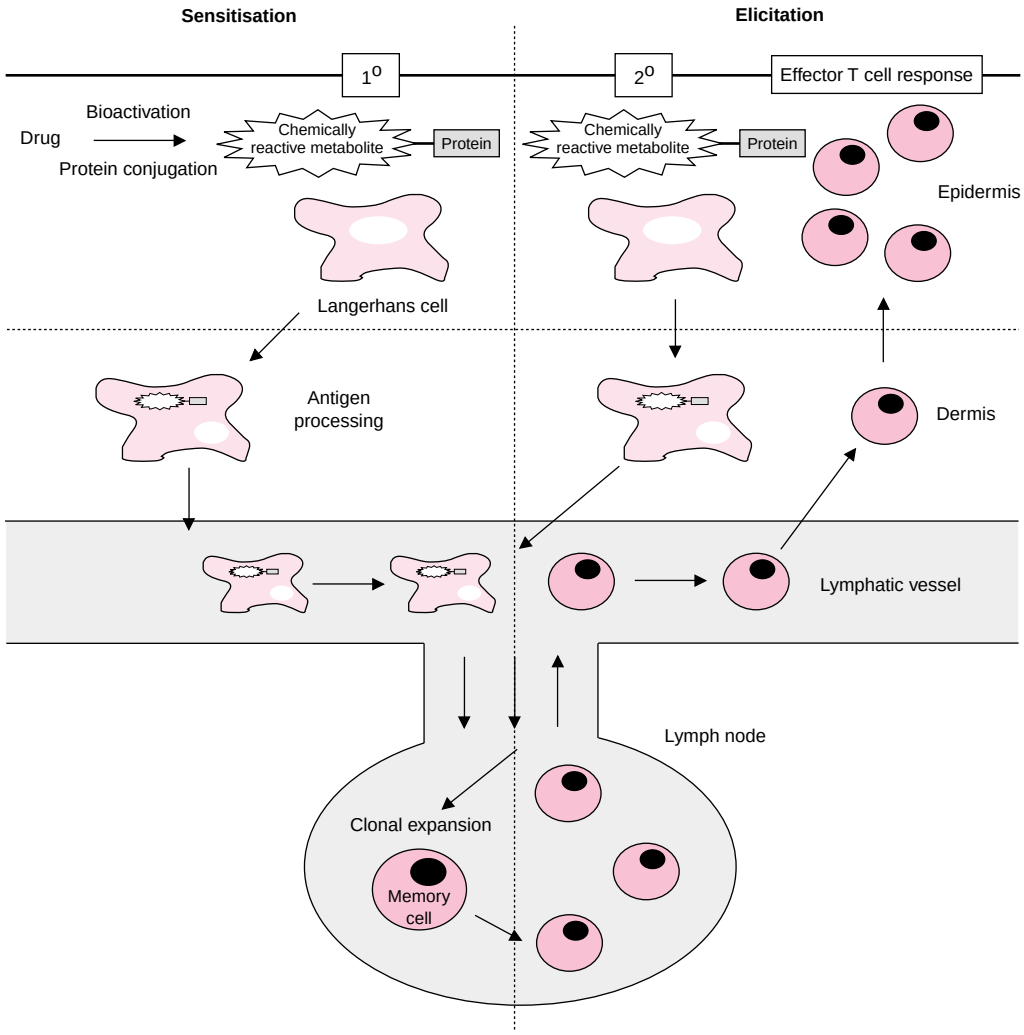

Fig. 4. The 3 stages of an immune response (chemical activation, immune recognition and elicitation of an immune response) following recognition of a cutaneous antigen.

modified proteins. Thus, the mechanism by which antigen presenting cells recognise drug-modified proteins (and foreign proteins in general) remains problematical and requires further investigation. This may become irrelevant, however, if one considers recent studies describing an alternative mechanism of immune recognition which occurs in the absence of protein haptenation and antigen processing.[66,67,113-116] In these *in vitro* experiments, proliferation of fresh and cloned T cells occurred in the absence of any apparent drug metabolism (table IV). Three lines of evidence were used in concluding that the parent drug can stimulate T cell proliferation in the absence of antigen processing. First, a simple washing step prevented proliferation, indicating that drugs, traditionally thought to

require metabolism and covalent binding to proteins to stimulate an immune response, are presented in the absence of a stable covalent bond.[66,113,114] Secondly, fixed antigen presenting cells that are unable to process peptide antigens (e.g. tetanous toxoid) present drugs such as sulfamethoxazole and lidocaine (lignocaine).[113,114] Finally, the kinetics of drug-specific T cell receptor downregulation, which are a sensitive measure of immune recognition, are similar to the recognition of pre-processed peptide antigens (i.e. less than 90 minutes).[114] Involvement of antigen presenting cells in the direct recognition of drugs has been demonstrated by MHC-restriction experiments using partly or unmatched antigen presenting cells.[113] From these observations the authors propose that drugs bind to the MHC complex of antigen presenting cells, or a peptide embedded within, in a noncovalent fashion. The nature of the binding remains unknown; however, the interaction is sufficiently stable to stimulate a cellular immune response. Figure 5 outlines proposed mechanisms of drug recognition and T cell presentation.

It is possible that each pathway is involved in drug hypersensitivity reactions, the antigen processing pathway being more important for elicitation of an immune response, while the processing-independent pathway may be more important for elicitation of allergic reactions in pre-sensitised individuals. However, this is unknown at present and requires further investigation. To evaluate the relevance of these novel concepts, a more detailed structural analysis of immune recognition is required using: (i) other drugs associated with a high incidence of hypersensitivity (e.g. anticonvulsants); and (ii) a sample size with sufficient power to investigate inter-individual variations in the immune response. Chemical methodology (e.g. high performance liquid chromatography and liquid chromatography-mass spectrometry) should also be used to determine the role of drug metabolism within these *in vitro* test systems. An essential step forward would be an x-ray crystallographic structure of the antigen presenting cell-drug-T cell interaction.

4. Induction of Drug Hypersensitivity: The Role of Cellular Metabolism and Antigen Processing

Antigen processing is the mechanism by which antigen presenting cells breakdown protein antigens to simple peptides prior to T cell presentation. MHC molecules, expressed on all antigen presenting cells, control the presentation of processed antigens (MHC-restriction).[117] It is widely accepted that T cells are crossreactive for a number of structurally unrelated antigens and such crossreactivity enables the immune system to respond appropriately to almost all known antigens.[118]

MHC molecules are composed of multiple immunoglobin domains.[119] The most distal portions are polymorphic and account for the genetic variability in the immune response. This portion of the MHC molecule is folded into a peptide binding groove that binds antigens.[120] Two distinct classes of MHC molecule present antigenic peptides to T cells.[121,122] MHC class I molecules are found on all cells and present antigens to CD8+ T cells. They preferentially bind peptides of 8 to 10 amino acids. In contrast, MHC class II molecules are found only on professional antigen presenting cells (B cells, macrophages, Langerhan's cells and other dendritic cells), and present longer peptide antigens (13 to 17 amino acids) to CD4+ cells.[98-102] Interferon-γ (IFNγ) upregulates MHC class I and II molecules

Table IV. Evidence for the direct, metabolism and processing-independent T cell recognition of drugs[114,115]

1. Drugs stimulate freshly isolated mononuclear cells and T cell clones in the apparent absence of drug metabolism
2. A simple washing step abolishes the proliferation of T cells, indicating that the drug was not covalently bound
3. Glutaral (glutaraldehyde)-fixed antigen presenting cells, which are unable to process protein antigens, can present drugs to specific T cell clones
4. The speed of drug recognition (approximately 1-2 minutes) as measured by Ca^{2+} influx and T cell receptor downregulation, suggests an immediate recognition of the drug without the requirement of metabolism and processing

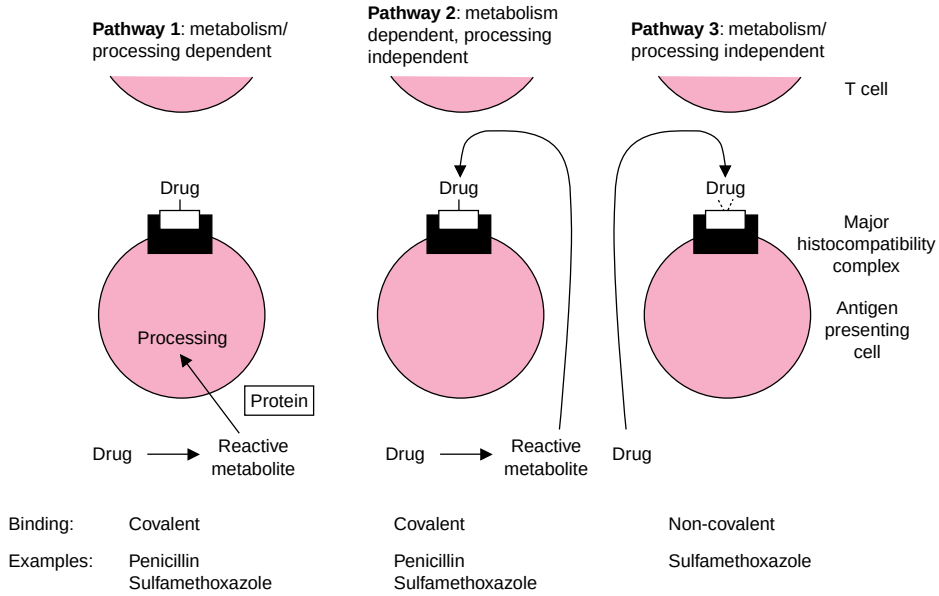

Fig. 5. Mechanisms of drug presentation to T cells.[114]

on antigen presenting cells[122] and renders target cells more susceptible to T cell mediated killing.[67]

Mature T cells are distinguished by the MHC-specificity of their antigen receptor. T cell receptor specificity occurs during positive and negative selection in the thymus.[123] CD4+ and CD8+ cells must express both the T cell receptor (CD3+) and the appropriate CD4+/CD8+ co-receptor for an efficient interaction. The T cell receptor itself is a broadly reactive molecule and the MHC class to which a cell is restricted is a function of the CD4+ or CD8+ accessory molecule.[124] T cells recognise both the modified peptide and the MHC molecule through complimentary interactions involving ionic, dipole and hydrophobic bonds. Presentation of a processed antigen in the presence of co-stimulation leads to the production of optimal levels of interleukin (IL)-2, upregulation in the expression of the IL-2 (CD25+) receptor and stabilisation of the T cell-antigen presenting cell interaction.[125,126] The absence of a co-stimulatory signal can result in tolerance and the T cell may undergo apoptosis.[127] The nature of an immune response is governed by differentiation of T cells into T helper-1 (T_H1), T helper-2 (T_H2), T cytotoxic-1 (T_C1) or T cytotoxic-2 (T_C2) subsets. Differentiation is dependent upon the antigen fragment presented, the T cell and the cytokine environment. T_H1 and T_C1 cells mediate cytotoxicity and local inflammatory reactions, whereas T_H2 and T_C2 cells are more effective at stimulating B cell dependent antibody production.

Antigen processing can originate inside or outside the antigen presenting cell. Exogenous antigens are presented on MHC class II molecules for recognition by CD4+ cells (e.g. bacteria and environmental allergens such as drugs or their metabolites), while endogenous antigens are presented on MHC class I molecules for recognition by CD8+ cells (e.g. viral, tumour associated antigens and lipophilic haptenating drugs).[128,129] The chemical reactivity and physico-chemical properties of a drug determine which MHC molecule presents the drug-protein antigen and the type of T cell response. Penicillin and dinitrofluorobenzene derivatives react directly with proteins and cause both CD4+ and CD8+ responses.[25] Alternatively, antigens derived

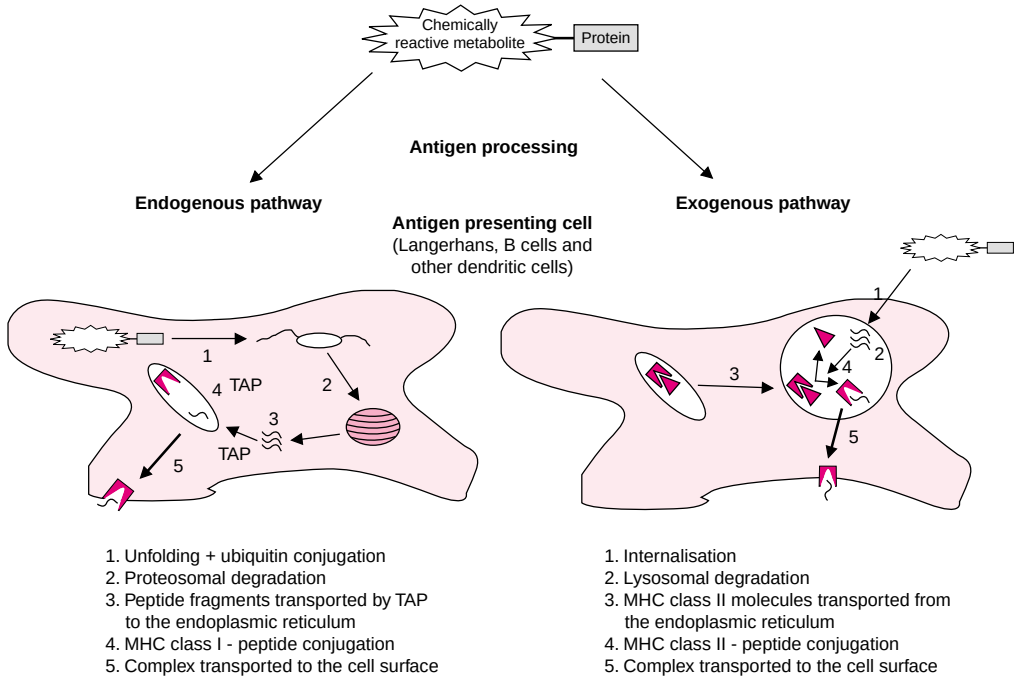

Fig. 6. The cellular and sub-cellular mechanisms involved in exogenous and endogenous antigen processing.[98] **MHC** = major histocompatibility complex; **TAP** = transporter associated antigen protein.

from propylthiouracil, which is metabolised by enzymes located on the exogenous pathway of antigen processing, are presented to CD4+ T cells bound to MHC class II molecules.[130] Similarly, metabolites generated extracellularly (compared with myeloperoxidase catalysed reactions)[35] are presented to CD4+ cells. Lipophilic drugs that cross the cell membrane tend to haptenate intracellular proteins and present to CD8+ cells via MHC class I molecules. Drugs such as sulfonamides and aromatic anticonvulsants are metabolised intra- and extracellularally, conjugate endogenous and exogenous proteins and have been shown to activate both CD4+ and CD8+ cells.[61,113,114]

The cellular and sub-cellular mechanisms involved in exogenous and endogenous antigen processing have been reviewed in detail (fig. 6).[98,99,102] Here we present a brief overview of antigen processing in the context of drug hypersensitivity. Exogenous antigens are recognised by cell surface receptors on antigen presenting cells. They are internalised by phagocytosis, endocytoplasmic vacuoles fuse with lysosomes and the antigenic protein conjugate is degraded into peptides by digestive enzymes in the acid milieu. MHC class II molecules, synthesised in the endoplasmic reticulm, are transported to the lysosome where they fuse with the processed antigen.[131] To increase the stability and prevent the binding of non-antigenic peptides, MHC class II molecules are transported with a membrane protein bound noncovalently to the antigen binding site.[132] A di-leucine moiety on the cytoplasmic surface assists transport and lysosomal accumulation.[133] The protein is cleaved by acid proteases, leaving a small embedded peptide. Such peptides [called class II associated invariant chain peptides (CLIP)] remain buried deep in the MHC binding domain.[134] The processed antigenic peptide replaces the bound

CLIP, a reaction catalysed by the MHC-encoded heterodimer HLA-DM.[135] HLA-DM controls the rate of peptide interchange by the disruption and/or distortion of conserved hydrogen bonds in the MHC binding pore.[136] The processed, MHC-restricted antigen is then exported to the cell surface for presentation to specific receptors on CD4+ cells.

In contrast, MHC class I molecules do not require a protein to prevent the binding of non-antigenic peptides. Endogenous antigens derived from cytoplasmic proteins are conjugated with ubiquitin and degraded into fragments by the proteosome complex. Processed peptides then associate with transporter associated antigen protein (TAP) and are transported to the endoplasmic reticulm.[137] Empty MHC class I molecules associate with the TAP-peptide conjugate, TAP is released and the peptide-conjugated class I molecule is transported to the cell surface.[138] MHC class I molecules present processed antigen to specific CD8+ cells. The cells become cytolytic and kill cells that express nonself antigens.[98,99]

5. Elicitation of Drug Hypersensitivity

5.1 The Role of Cytokines

Cytokine production initiates and sustains an immune response. In the presence of IL-1, IL-12 and the appropriate receptor and co-stimulatory interactions, T cells proliferate, upregulate the expression of IL-2 and secrete further cytokines which determine the characteristics of the immune response (fig. 7). IL-12, released by macrophages and natural killer cells, is required to stimulate CD8+ cells, while IL-1 induces a CD4+ response. A number of co-stimulatory ligand–receptor interactions have also been identified. The best characterised interaction involves B7.1 and B7.2 molecules that interact with T cells expressing the CD28+ receptor. CD28+ stimulation upregulates the production of IL-2.[139,140] The interaction of B7.1 and B7.2 molecules with CTLA-4 provides inhibitory signals.[141] Differential expression of CD28+, CTLA-4 and B7 serves to control the stimulatory response. CD28+ is expressed on resting and activated T cells, while CTLA-4 and B7 molecules are expressed on activated T cells and activated antigen presenting cells, respectively.[142-144] Additional signals that occur during antigen recognition include the CD2+, CD30+ and CD40+ receptor/ligand interactions.[145-147] Although less well defined, these co-stimulatory signals upregulate the expression of adhesion molecules [lymphocyte function-associated antigen (LFA)-1, intercellular adhesion molecule (ICAM)-1, LFA-3] and cytokines.[148-151]

Humoral and cellular responses represent the 2 major types of specific immunity. CD4+ cells control the nature of the immune response, while CD8+ cells and antibodies produced by B cells mediate cellular and humoral responses, respectively. CD4+ T cells contain the majority of T helper (T_H) cells and direct the type and severity of the response, while CD8+ cells are generally cytotoxic (T_C) or suppressor.[152,153] It is important to note, however, that segregation of CD4+ and CD8+ cells into T_H and T_C populations is not absolute. Some T_C are CD4+, while some T_H cells are CD8+.[98] These characteristics portray how both T cell subsets perform similar functions using overlapping mechanisms. Both subsets produce a similar pattern of cytokines [CD4+, T_H1 (IL-2, IFNγ, tumour necrosis factor-β) and T_H2 (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13); CD8+, T_C1 (IL-2, IFNγ) and T_C2 (IL-4, IL-5, IL-6, IL-9, IL-10)] and mediate a cytotoxic response when stimulated with an antigen.[154-156] In general, CD4+ cells produce higher levels of IL-4 and IL-5 which are needed to stimulate the B cell dependent production of antibodies, while CD8+ cells produce high levels of the inflammatory cytokine IFNγ.[157] IFNγ transforms monocytes into macrophages and induces the production of the chemokine IFN-inducable protein-10 which is responsible for attracting infiltrates of leucocytes.[158]

Recent studies demonstrated that T cell help for cytotoxic T cells can be mediated through an antigen presenting cell bridge.[159,160] The authors suggested that cytokines from T_H cells stimulate CD40 molecules on antigen presenting cells, which in turn provides signals to CD8+ cells. Activated antigen presenting cells express B7 and interact with naive

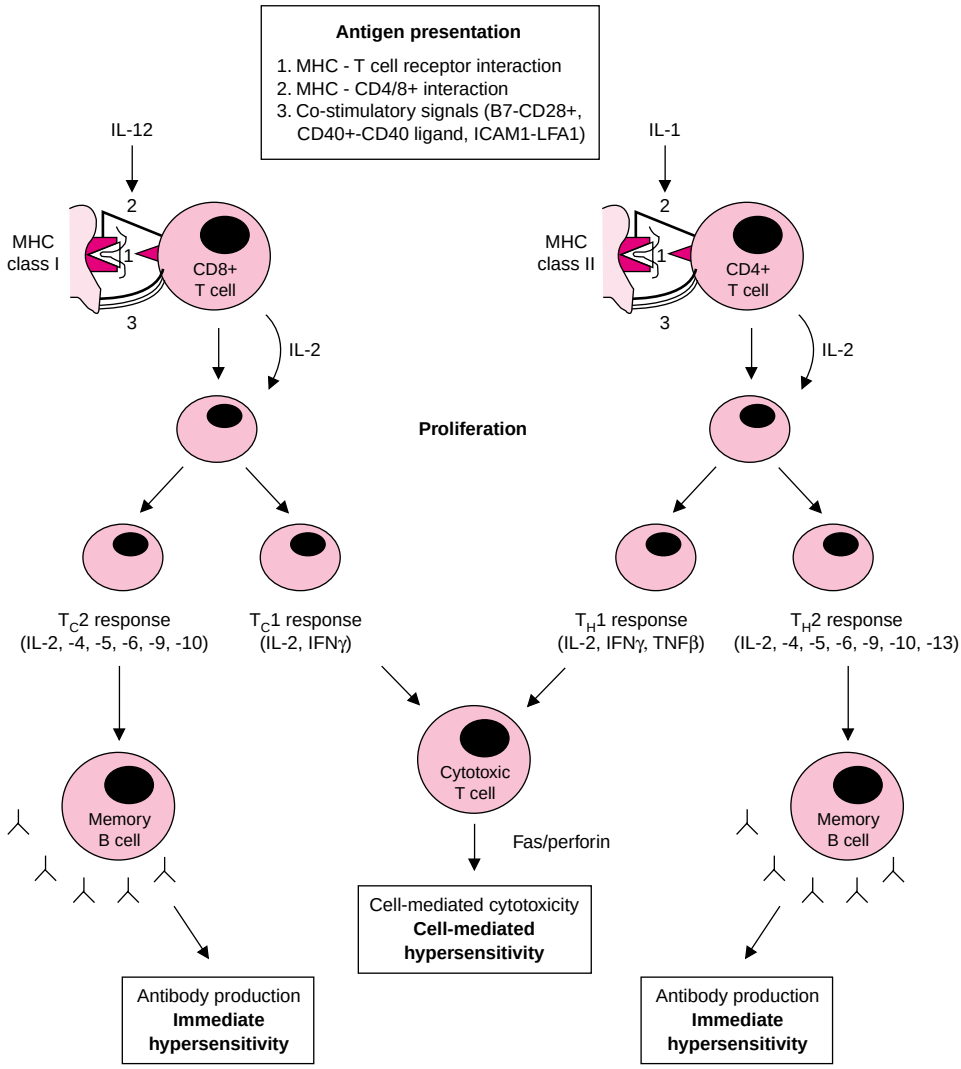

Fig. 7. The role of cytokines in the development of a polarised immune response. **ICAM** = intercellular adhesion molecule; **IFN**γ = interferon-γ; **IL** = interleukin; **LFA** = lymphocyte function-associated antigen; **MHC** = major histocompatibility complex; **T_C1** = T cytotoxic-1; **T_C2** = T cytotoxic-2; **T_H1** = T helper-1; **T_H2** = T helper-2; **TNF**β = tumour necrosis factor-β.

CD8+ cells without the direct need for CD4+ T cell help. Similarly, a non-activated antigen presenting cell has the potential to inhibit T_H activation in the absence of further signals. A negative feedback mechanism is thought to control which is the dominant pathway.

5.2 The Polarised T Cell Response

T_H1 cells promote the activation of macrophages and cell-mediated immunity.[161] In contrast, T_H2 cells provide help for the humoral immune response by promoting IgG to IgE class switching and through

stimulation of mast cells and eosinophil growth factors.[162] Cells that produce both cytokine patterns (T_H0) are capable of stimulating both types of response.[163] T_H1 responses eradicate invading pathogens and are responsible for the majority of immune-mediated adverse drug reactions, while T_H2 cells mediate T_H1 responses and suppress excessive or inappropriate responses.[100,164,165] Two activation markers, lymphocyte activation gene-3 (LAG-3) and CD30+, are preferentially associated with polarised T_H1 and T_H2 responses, respectively.[100] LAG-3 is strongly upregulated by IL-12 and its expression correlates with IFNγ.[166,167] CD30+, a member of the tumour necrosis factor receptor family, is expressed and released by activated T_H2 cells.[168]

Although the function of T_C1 and T_C2 cells are less well defined, both sub-populations are cytolytic, and kill target cells by apoptosis or necrosis, by the Fas and perforin pathways, respectively.[169,170] T_C2 cells, however, have been shown to lose their cytolytic activity and provide B cell help.[171] It has also been hypothesised that T_C2 cells act as suppressor cells through the production of helper cytokines.[172]

Route of administration and dose of an antigenic moiety, the genetic constitution of an individual and site of antigen presentation determine the T_H cytokine profile.[100,173,174] The type of response is also dependent on the antigen presenting cell and extent of co-stimulation. B7.1 promotes T_H1 and T_H2 responses equally, while B7.2 and CD40 ligand preferentially signal T_H2 and T_H1 responses, respectively.[175] Despite these observations, the most important determinant of T_H1 and T_H2 differentiation is the availability of cytokines from the innate immune system.[176,177] IL-12, synthesised by antigen presenting cells during antigen presentation, is the dominant cytokine for the induction of T_H1 responses, while IL-4 and prostaglandin E2 (released from antigen presenting cells, mast cells, memory T cells and naive T cells) are critical for the development of a T_H2 response and controlling the T_H1 response.[178-180] The anti-inflammatory cytokine IL-10, which has been shown to inhibit T cell proliferation, also regulates the development of T_H1 responses by blocking IL-12 release.[181] Recently, Cavani et al.[182] demonstrated the presence of nickel specific IL-10 producing CD4+ cells that regulate whether an antigen induces hypersensitivity or an immune response in the absence of clinical symptoms. The role of regulatory T cells in drug hypersensitivity, however, is unknown and requires further investigation.

Glutathione and the intracellular redox balance are believed to play an important regulatory role in the early interactions between T cells and antigen presenting cells. Chronic depletion of glutathione inhibits processing and presentation,[183] while a mild or moderate depletion influences the T_H1/T_H2 cytokine balance.[184] Peterson et al.[184] demonstrated that a 20 to 30% depletion of glutathione inhibits T_H1 (IL-12, IFNγ) associated cytokine production and favours T_H2 (IL-4) responses. These findings were attributed to short term depletion of glutathione from antigen presenting cells, and not T cells. It is possible that drugs (chemically reactive metabolites) or disease, which have been shown to deplete intracellular glutathione,[74,86,185,186] may also influence the type of response by modifying T_H1/T_H2 cytokine patterns. The mechanism(s) by which T cell responses are modulated, however, remain unknown and require further investigation. Such studies may elucidate the relationship between an increased frequency of drug hypersensitivity and human diseases such as AIDS.

6. The Danger Hypothesis of Immune Recognition

Our understanding of drug hypersensitivity is based largely on the hapten theory of immune recognition; the onset of toxicity is thought to involve drug bioactivation, covalent binding to proteins, followed by uptake, antigen processing and T cell proliferation.[5,96] The way in which the immune system recognises antigenic moieties has been enhanced by recent chemical and immunological perspectives. Curtsinger et al.[187] proposed that 3 independent signals are required to stimulate a full immune response. Signal 1 is the interaction between a MHC-

restricted antigen and the T cell receptor. Signal 1 itself does not induce an immune response if delivered alone and in the absence of secondary signals, tolerance supersedes.[188] Signal 2 represents a series of proinflammatory cytokines that act indirectly on antigen presenting cells by upregulating the expression of co-stimulatory molecules. Signal 3 involves polarising cytokines, released from the innate immune system, that act directly on T cells.[187] A serial triggering pathway may be involved in the full activation of an immune response. For example, T cell activation requires only signals 1 and 2 at high antigen concentrations; however, signal 3 acts as an adjuvant at low antigen concentrations and thus enhances the response.[187,189]

The danger hypothesis, initially described by Matzinger,[188] and recently reviewed,[5,109,190] has increased our understanding of the mechanism(s) of immune recognition (fig. 8). This 'alternative' or 'additive' hypothesis states that the immune system responds to most antigens with tolerance, and only in the presence of a 'danger signal' will presentation of an antigen result in an immune response. The danger hypothesis, based on the laws of lymphotics,[186] defines the role of secondary and tertiary co-stimulatory signals. The lymphotic laws state: first, a cell will die if it receives signal 1 in the absence of signal 2; and secondly, T cells will only accept signal 2 from antigen presenting cells. These laws stand for naive and memory T cells; however, naive T cells only receive signal 2 from professional antigen presenting cells. To control the immune response, special conditions apply to effector cytotoxic T cells and plasma cells. They rapidly lose the requirement for co-stimulation, but will die or revert to a resting state after a short period of time.

The laws of lymphotics accurately define the cellular aspects of an immune response; however, the mechanism(s) that upregulate 'danger' are less well defined. Danger signals may originate from stressed cells (i.e. transcriptional activation), in the presence of tissue destruction (i.e. necrosis) or, in theory, may be any molecule that is not normally found outside cells (e.g. mitochondria or DNA). Heat shock proteins may be of particular importance.[191] The expression of heat shock proteins is upregulated in cells undergoing stress or those damaged or inflamed. In contrast, van Eden et al.[192] have shown that upregulation of heat shock proteins, as a consequences of stress, provides an immunoregulatory response that contributes to the maintenance of self-tolerance. Clearly, expression of heat shock proteins, and their role in drug hypersensitivity requires further investigation.

It is possible that a drug could provide signal 1, 2 and 3. Alternatively, drug-protein conjugation may occur without co-stimulatory signals, especially when the drug is administered at low dosage regimens. This would explain why dosage regimens that start with a low dose of drug are less likely to lead to hypersensitivity.[193,194] Drugs may cause signal 2 (cell stress/necrosis) in the absence of signal 1. Liberation of intracellular contents generate antigens and thus may produce an autoimmune reaction.[195] The amount of reactive metabolite generated determines the extent of tissue damage and whether a severe immune response would ensue. Drugs may also provide signal 1 and an infection or surgical trauma signal 2 and/or 3.

Direct evidence to prove or disprove the danger hypothesis remains scant. It should be noted that issues such as blood transfusions seem to make this theory less universal than it was initially thought to be. The transfusion of blood involves the same danger – tissue injury during catheter insertion – for AB versus type O blood, but the immune consequences for the individual with type O blood are quite different. Hanahan and colleagues[196-198] have presented data concerning the large T cell antigen expressed in transgenic mice that makes the same point. On the positive side, dendritic cells (the most potent antigen presenting cell), are activated by endogenous signals received from cells that are stressed, virally infected or killed necrotically, but not healthy cells or those dying apoptotically.[199] Uptake of necrotic cells results not only in antigen presentation on the surface of cells, but also in the activation of the dendritic cell to express co-stimulatory molecules that are necessary for T cell ac-

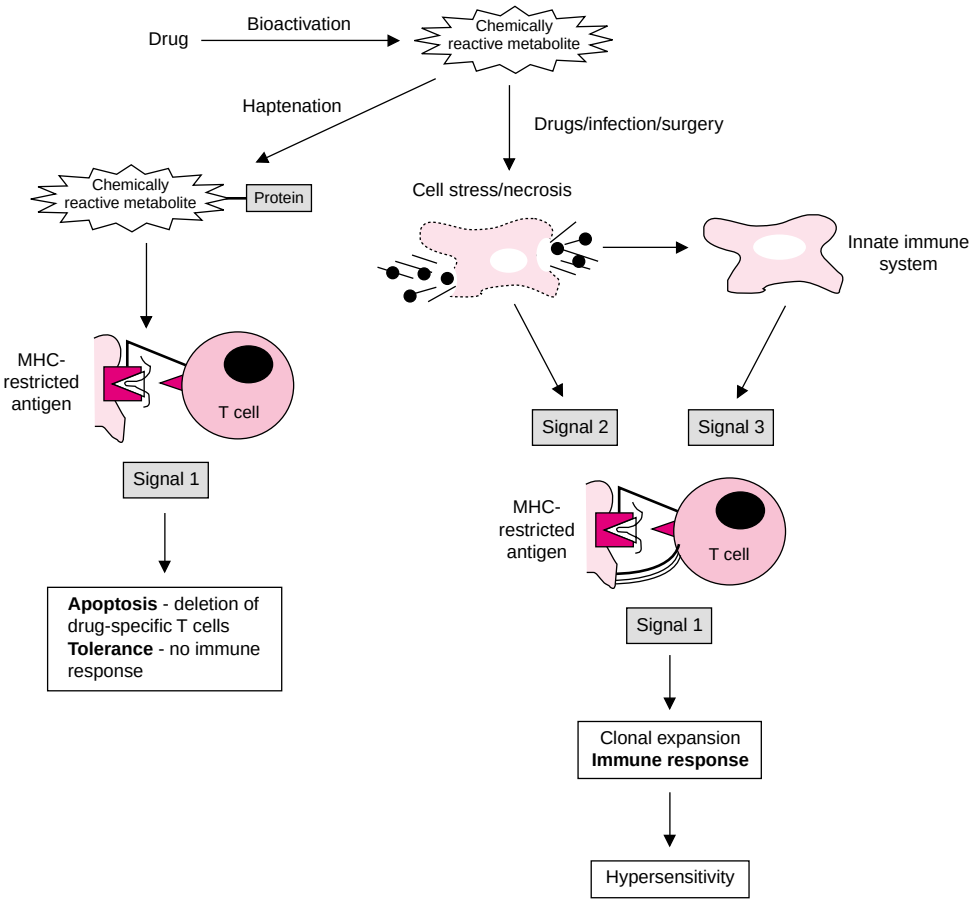

Fig. 8. Application of the hapten hypothesis, danger hypothesis and serial triggering model to drug hypersensitivity. **MHC** = major histocompatibility complex.

tivation. In contrast, apoptotic cells do not initiate co-stimulation.[200]

Work by Fadok et al.[201] demonstrated that engagement of the phosphatidylserine receptor, which is expressed on apoptotic cells, provides the primary signal linking uptake of apoptotic cells to the production of anti-inflammatory cytokines and immune tolerance. These observations may be consistent with: (i) case reports where hypersensitivity to sulfasalazine was associated with the reactivation of human herpesvirus 6;[202,203] and (ii) an increased prevalence of drug hypersensitivity in HIV positive patients.[94,95,204,205] However, it is likely that the infection – danger hypothesis is only one component of a complex and multi-factorial predisposition to the increased frequency of drug hypersensitivity in virally-infected patients.

7. Conclusions and Future Perspectives

An effective MHC-restricted antigen presenting cell – T cell interaction, in the presence of the appropriate co-stimulatory signals, initiates an immune response. Clonal expansion and cytokine production generate antigen-specific effector cells and sustain the response. Figure 9 details our current understanding of drug-induced immune modulation

using sulfamethoxazole and penicillin as examples. Sulfamethoxazole, used in combination with trimethoprim (cotrimoxazole), is the preferred treatment for *Pneumocystis carinii* pneumonia in patients with HIV-infection. Sulfamethoxazole is associated with mild urticarial rashes and severe reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis, which occur as part of a generalised hypersensitivity reaction involving one or more internal organs.[9] Hypersensitivity is presumed to involve drug metabolism and the formation of a drug-protein conjugate. Following antigen recognition, the drug-modified protein is processed and presented on MHC class I and/or II molecules to T cells (signal 1).[5,96] Danger signals, which determine whether immune recognition results in tissue damage, rather than tolerance, may originate from the drug (or chemically reactive metabolite) itself, or alternatively they may be completely independent of the drug, and be a host factor, such as viral or bacterial infection.

In the liver, sulfamethoxazole is metabolised to a hydroxylamine.[206] Sulfamethoxazole hydroxylamine circulates in blood and tissues and is excreted in human and rat urine.[207,208] Further (auto)oxidation yields a nitroso compound that can haptenate sulfydryl-containing proteins, including the surface of viable lymphocytes.[86] These observations, identification of sulfamethoxazole-substituted hepatic proteins *in vitro*, sulfamethoxazole-substituted serum proteins and anti-sulfamethoxazole antibodies in patient sera, and CD8+ dermal T cells that proliferate in response to microsome-generated sulfamethoxazole metabolites lend weight to the involvement of drug metabolism and the immune system in the pathogenesis of drug hypersensitivity.[85,107,209,210] Despite this, sulfamethoxazole has been used as a paradigm to define the metabolism and processing-independent pathway of immune recognition of drugs by T cells.[66,67,113-116] In these studies, the majority of sulfamethoxazole-specific T cell clones were CD4+ and secreted high levels of IL-5 and IL-4. Some of the CD4+ cells, and even fewer of the CD8+, cells gave a clear T_H1 cytokine pattern (high IFNγ, low IL-4, IL-5). However, these data must be regarded with some caution until the cytokine profile of further patients with hypersensitivity has been determined.

Penicillin can cause hypersensitivity reactions that fit all 4 categories of the Coombs and Gell classification. The β-lactam ring of penicillin is a target for nucleophilic attack by free lysine groups of proteins in the absence of drug metabolism (fig. 3).[15,72] Lymphocytes from patients hypersensitive to penicillin proliferate *in vitro* in response to the appropriate β-lactam antibiotic. T cells isolated from these cultures were of the CD4+ and CD8+ phenotype and secreted heterogeneous patterns of cytokines.[18] Since the β-lactam ring is the primary antigenic moiety, there is virtually complete cross-reactivity among penicillins.[211] The main difference between the presentation of penicillin and sulfamethoxazole is that penicillin needs to covalently modify proteins to become immunogenic, while sulfamethoxazole may be presented in the presence or absence of a haptenated protein. Recent studies analysing the proliferative response of T cell clones from patients with sulfamethoxazole hypersensitivity demonstrate that the great majority of clones are specific for noncovalently bound sulfamethoxazole and only a small fraction responded to the chemically reactive nitroso metabolite.[212] However, further combined chemical and cellular investigations are required to determine the role of drug metabolism and antigen processing in both the primary sensitisation and restimulation of T cells from individuals with hypersensitivity.

An increased understanding of the chemical and immunological mechanisms of drug hypersensitivity is important for several reasons: first, it may allow the development of screening methods early in drug development, which are predictive of the immunogenic potential of new chemical entities. Secondly, it may allow a reduction in the incidence of drug-induced hypersensitivity reactions by allowing prediction of susceptible individuals. In this regard, although most studies to date have concentrated on single genes, such as HLA DR4 with hydralazine-induced systemic lupus erythematosus,[213] the completion of the human genome proj-

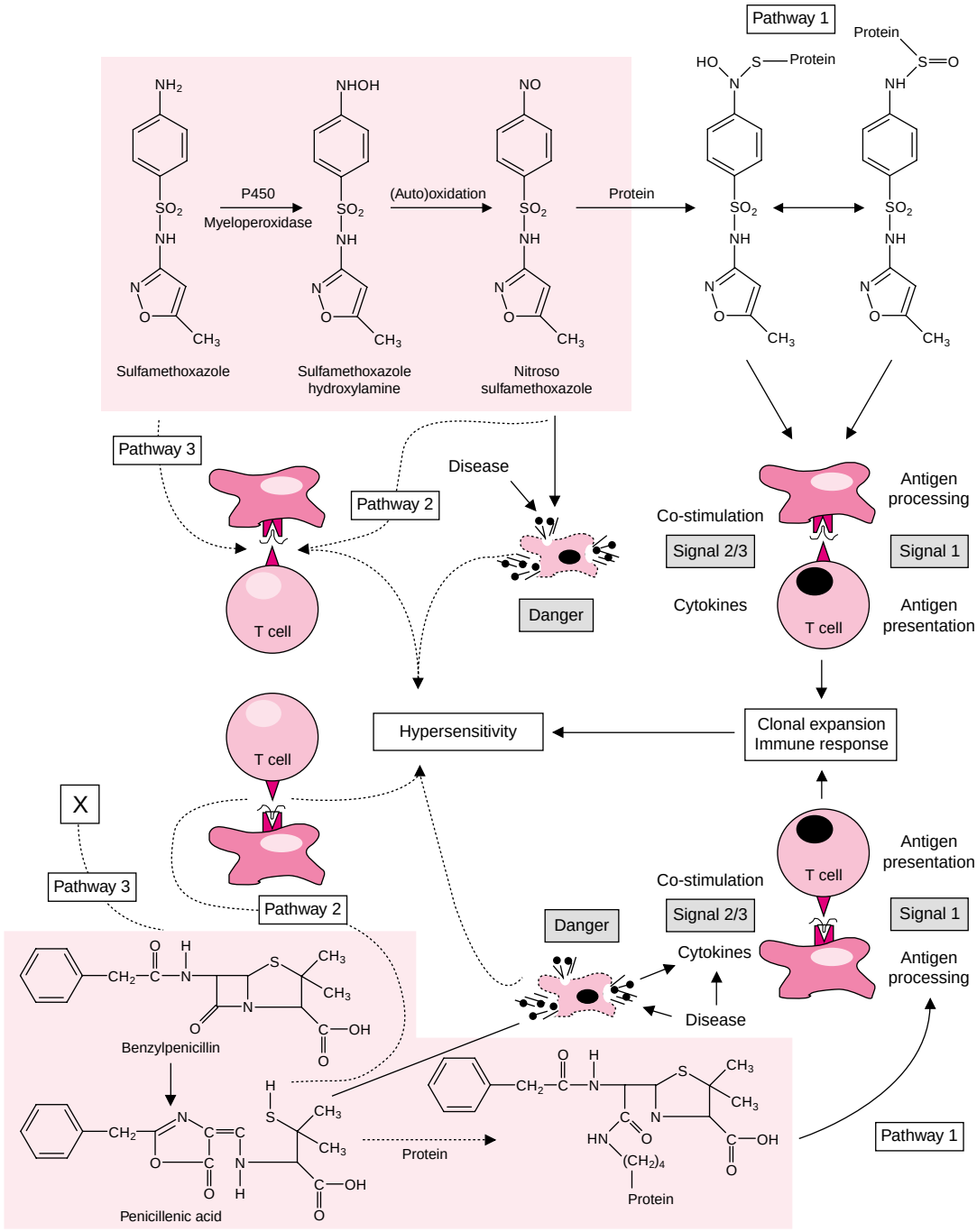

Fig. 9. The initiation and propagation of immune responses elicited by sulfamethoxazole and penicillin.

ect, together with the identification of single nucleotide polymorphisms in the genome, will allow genetic profiling through the emerging science of pharmacogenomics.[214] Thirdly, elucidation of mechanisms through identification of the protein targets in hypersensitivity will allow the development of diagnostic tools so that the clinician is better equipped to decide which of the multiple drugs that the patient was taking was responsible for the reaction.

Lastly, a knowledge of mechanisms will allow the development of better treatment strategies, or a better understanding of currently used therapies. With respect to the latter, a good example has emerged recently in relation to the treatment of toxic epidermal necrolysis (TEN). TEN, an immune-mediated condition invariably caused by drugs, has been treated empirically with corticosteroids and thalidomide, sometimes with disastrous consequences.[215,216] Intravenous immunoglobulins have also been used in the treatment of TEN; a recent study has shown that these immunoglobulins inhibit CD95 (Fas receptor)-mediated keratinocyte death.[217] The identification of a method whereby the pathogenetic events in TEN can be blocked promises the development of more specific (and more efficacious) therapies in a condition associated with high mortality.

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